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CONTENTS

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FLAME PHOTOMETRY

ALMA J. SEGER, B.S., M.T. (ASCP)
EDWARD J. VAN LOON, Ph.D., and MERLE R. LIKINS, M.S.

The flame photometer is an instrument designed primarily to facilitate the rapid and accurate analysis of such metallic elements as sodium and potassium, which have intense and characteristic flame spectra. It measures the intensity of the light emitted by the elements when excited in a flame.

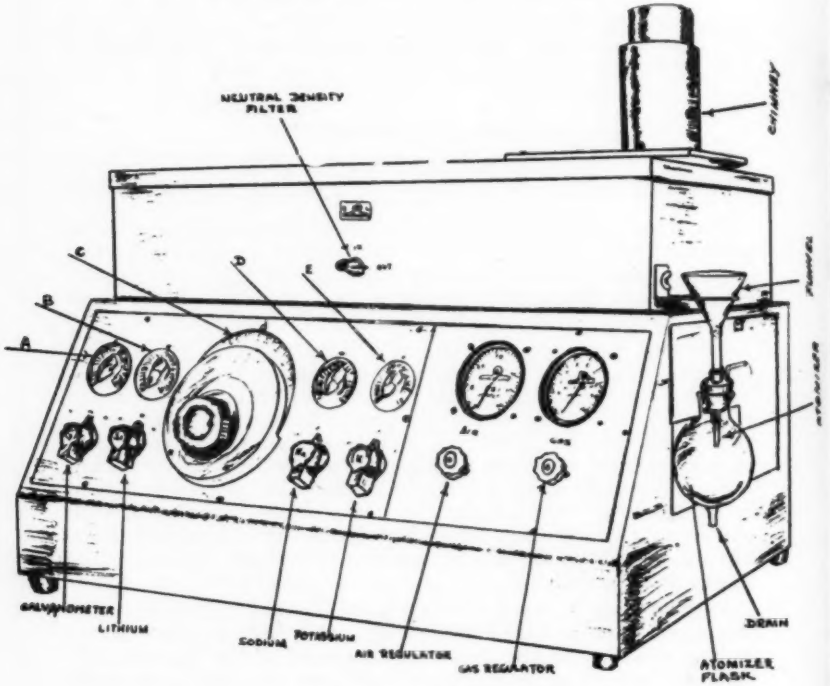
Although this science is nearly a hundred years old, until a few years ago very little actual quantitative work was done with it. The earliest report of it in literature is in 1860 when Kirchoff and Bunsen used this principle for the detection of various elements. In 1915 and 1920 an account of a crude type of flame analysis for the presence of water soluble potassium was published. It consisted of a platinum wire loop being dipped in an unknown solution and held in a flame, viewing it with blue spectacles against a white background in a darkened room. The amount of potassium present was calculated by noting the length of time that the potassium flame persisted.[1]

A great step forward in the perfection of flame photometry was made when in 1929 Lundegardh, using high pressure acetylene-air mixtures as the flame source, dispersed the emitted light through a quartz prism onto a photographic plate and determined the concentration of the element under study from measurement of the density of its spectral lines.[2] Later this photographic procedure was eliminated by a direct reading flame photometer which utilized phototubes to measure the intensity of the spectral lines.[3]

From the Medical Research Laboratory, Veterans Administration Hospital, Louisville, Ky.

Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

Today there are a number of companies marketing flame photometers, some of which are the Barclay, the Perkin-Elmer, and the Beckman. Basically, these photometers are similar, yet each has its own design and pattern for analysis. In our work at the Veterans Hospital we are using the Barclay instrument which is a filter-type instrument. Therefore, all the data, sources of error, and other findings presented are based on this instrument.



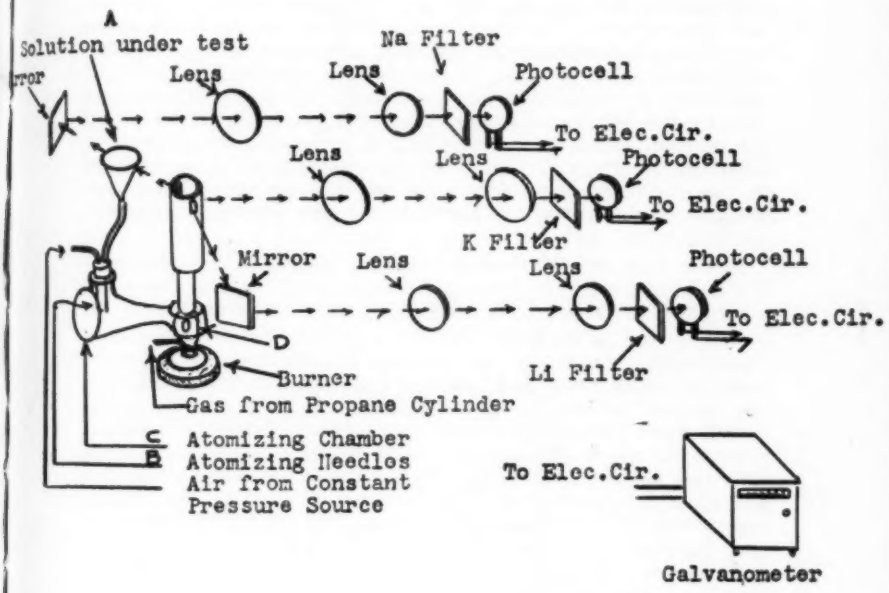
BARCLAY FLAME PHOTOMETER

Plate I

The Barclay Flame Photometer has been designed for the quantitative determination of the alkali metals, particularly sodium and potassium, and uses an air-propane flame as the means of excitation. When this flame is used only the alkali metals and alkaline earth constituents of the sample will emit light in appreciable amounts. This is particularly important,

since the fewer the number of constituents of any given sample which are excited, the more accurately can their light characteristics be isolated and measured.[4]

The sample to be analyzed must be brought into solution, and generally water is the preferred solvent medium, although some authorities say dilute ethanol is useful for serum determinations.



FUNCTIONAL DIAGRAM BARCLAY FLAME PHOTOMETER

Plate II

The diluted solution is poured into the funnel, and the flow of air through the capillary past the tip of the funnel atomizes the sample solution. The larger droplets of the solution condense on the inner walls of the atomizer chamber and run off to the drain, while a fine mist of the sample is aspirated into the mixing chamber at the base of the burner where it is mixed with the gas entering the burner.

The light from the flame passes to the optical system which consists of three channels, each of which is fitted with appropriate lenses, filters and a photocell. The light filters in any one channel are designed to pass only the characteristic radiation of one element, sodium, potassium or lithium. Thus, the photocell in Channel 1 receives only radiations due to sodium and is consequently designated the sodium photocell. In a similar manner the cells in Channels 2 and 3 receive light only from potassium and lithium, respectively, and are designated as the potassium and lithium cells. The light which strikes the photocell gives rise to an electrical impulse which is a function of the quantity of the respective metal in the sample. The electrical response of the photocell to the light may be thought of as an e.m.f. so that actually the photocell becomes a current generating device, and the magnitude of the current is a function of the amount of metal in the sample. This electrical response may be utilized in two different ways for measurement purposes. These are designated the Absolute Method and the Internal Standard Method.

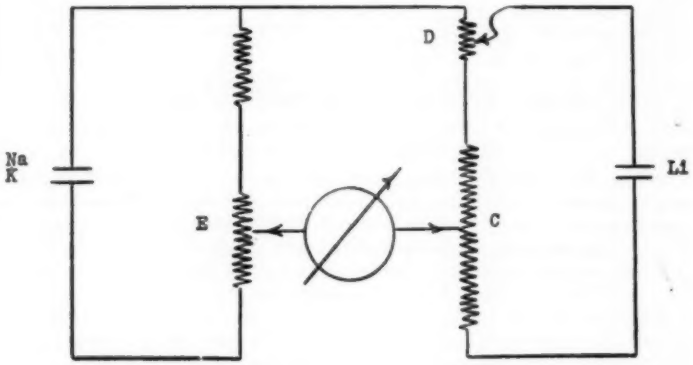
In the Absolute Method the current output of the photocell is measured directly by having it flow through the galvanometer, where the galvanometer is used as a micro-ammeter.

Among the prime requisites for successful quantitative and reproducible results by this method are the establishment of constant atomization and burning conditions of the flame, and the absence of interfering substances from the sample being tested. Each flame photometer has certain individual characteristics with which the operator must become familiar, in order to obtain satisfactory results. The optimum pressure of air and gas must be determined. This is accomplished by measuring transmittance at different pressures of air, keeping the gas pressure constant, and vice-versa. Viscosity influences the rate of atomization and thus the transmittance values. Although the Barclay Flame Photometer has been designed to reduce the former difficulties to a minimum there still remains the even more serious errors due to interfering substances in the sample. Glucose in a diabetic urine has a very great effect. It depresses transmittance value. Phosphates also act in this manner. Therefore, even though the Absolute Method can be, and has been, used for quantitative determinations, it is not recommended for such use. It is, however, a very rapid and easy method for qualitative and semi-quantitative work. Occasionally in pathological cases we use the Absolute Method just to get an approximation of the concentration of sodium or potassium which is often found to be extremely high or low.

In our research work, since it is of utmost necessity that we use a method by which we are able to get reproducible results

time and again, we have by-passed all the disadvantages of the Absolute Method and have adopted the Internal Standard Method. By doing this we eliminated all of the above mentioned inherent difficulties to a very high degree. In this method a fixed quantity of lithium, usually in the form of lithium sulfate, is added to the sample which is to be analyzed for sodium or potassium. Upon excitation in the flame, light is emitted by both the element being determined (sodium or potassium) and the internal standard lithium, and the ratio of the light intensities is measured.

The actual physical measurement involves a null point determination in which the electrical impulse of the sodium or potassium cell is balanced by that of the lithium cell. Plate III shows schematically the electrical circuit used to accomplish this. Here the sodium or potassium photocell and the lithium photocell act as a source of an e.m.f. to two potentiometer circuits which are opposing one another, and the galvanometer is used as a null instrument. (The rheostat "E", corresponding to dial "E" on front panel, may be used to vary the output of the sodium or potassium cell to the null circuit.)



SCHEMATIC DIAGRAM OF ELECTRICAL CIRCUIT
Plate III

The rheostat "D", corresponding to dial "D" on front panel, is to be used for small compensating adjustments which may be required from time to time due to changes in the characteristics of the lithium cell.

Once the proper settings of "E" and "D" have been chosen according to the characteristics of the particular cells being used and the concentration range of the unknown, they are left

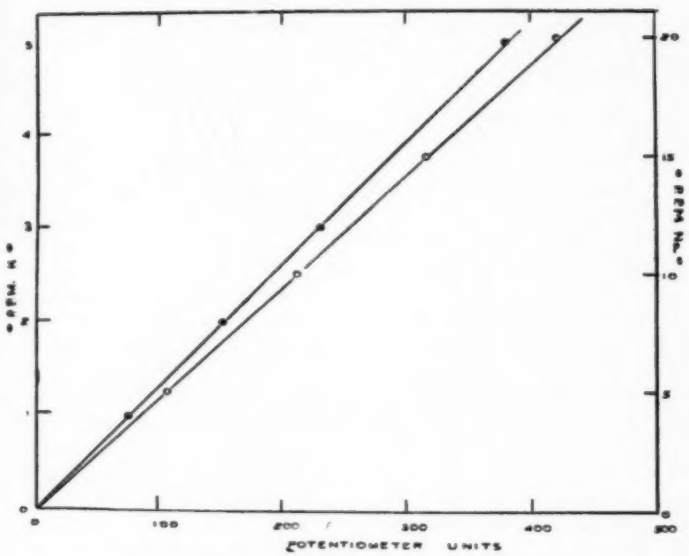
constant for any particular calibration. The actual measurements are made by use of the potentiometer "C". When an unknown is run through for determination, it will deflect the galvanometer from its zero setting, and the dial setting of "C" required to zero the galvanometer is a measure of the unknown. The actual value is obtained from a calibration curve.

The principle of the Internal Standard Method is that factors which influence the light intensity emitted by sodium or potassium will influence that emitted by lithium in the same manner and to the same degree, so that the ratio of the light intensities which is actually measured will remain constant.

We are now ready to calibrate our instrument. The first step is to determine the correct concentration of lithium necessary as an internal standard. By experimenting with different concentrations of sodium, potassium, and lithium, we found the optimum concentration of lithium for our instrument to be 100 p.p.m. for sodium determination and 300 p.p.m. for potassium determination. This concentration of lithium is added to each dilution of sodium and potassium on which a determination is to be made. The settings of "D" and "E" that were used above are maintained, the galvanometer switch is kept on "Int. Std.", the lithium switch is maintained on "Int. Std.", the sodium switch is thrown to the "Int. Std." and the potassium switch is in "Off" position for sodium calibration. For making the potassium calibration the latter switches are changed so that the potassium switch is on "Int. Std." while the sodium switch is "Off."

In each calibration the galvanometer is set on center zero while the first solution (0.0) p.p.m. of sodium or potassium is run through the funnel and the potentiometer "C" is on zero. The remaining standard solutions are successively introduced into the funnel and the potentiometer "C" is adjusted in each case until no current flows through the galvanometer; that is, it remains steady at center zero. The potentiometer reading obtained for each solution is then plotted versus the concentration, yielding the calibration curve as shown in Plate IV.

Now we will determine our unknown. After the specimen to be analyzed plus the necessary content of lithium sulfate is diluted, the solution is poured through the funnel and a balance point obtained as above by use of the potentiometer "C". The setting of the potentiometer should then be used to obtain the sodium or potassium content from the proper calibration curve. Since the calibration curve for the standard has a tendency to undergo slight changes during any one day's run, it is advisable for precise work to check one or two points of the curve several times during the day.



CALIBRATION CURVES FOR SODIUM AND POTASSIUM
Plate IV

It is necessary to keep the air pressure constant so that atomization of the sample will proceed at a uniform rate. With interferences minimized, we know from our experience with solutions of known concentration that we can analyze biological materials with a very low percentage error, usually around ±1 to 2% error. Tables I and II are examples of some of our work.

TABLE I
Recovery of Potassium

A. Unknown Urine Specimen	104.5 mgs.%
B. "A" plus 20 mgs.% K	125.0 mgs.%
C. "B" plus 20 mgs.% K	145.0 mgs.%
D. "C" plus 20 mgs.% K	165.0 mgs.%
E. "D" plus 20 mgs.% K	186.0 mgs.%
F. "E" plus 20 mgs.% K	208.0 mgs.%

TABLE II
Potassium Determinations on Pooled Urine Samples

Flame Photometer	Chemical Method
1.40 gm./24 hrs.	1.38 gm./24 hrs.
2.06 gm./24 hrs.	2.12 gm./24 hrs.

0.84 gm./24 hrs.	0.85 gm./24 hrs.
1.10 gm./24 hrs.	1.22 gm./24 hrs.
1.35 gm./24 hrs.	1.27 gm./24 hrs.
2.68 gm./24 hrs.	2.64 gm./24 hrs.

All authorities on flame photometry are agreed that this new analytical art is subject to numerous errors, some of which are understood and can be dealt with, whereas others remain obscure. These errors are in many and unexpected forms. For instance, handling soap powder in the laboratory may pollute the air for hours; tobacco smoke contributes potassium in considerable quantity, or air in a poorly ventilated room may become polluted from the chimney fumes of the photometer. We have our flame photometer on a table next to the hood, which we run while doing our determinations so we have just about ruled out the above errors. As is true in all sodium and potassium determinations, care must be used in the grade of filter paper used if you find it necessary to filter your specimen. Common filter paper may contribute sodium and potassium. Also, remember when inserting a glass stopper into a flask not to give it a grinding twist because in doing this you may release sodium and potassium to the solution. Be careful of new glassware containing a surface alkali. Remove this by soaking for several days in distilled water.

You may wonder why I continually spoke of dilution of samples to be tested and why the standard solutions are run at such low concentrations. This is because that at decreased concentrations the interference is also decreased even though the ratio of the interferent to the metal remains constant. It follows, therefore, for minimum interference from foreign substances, flame photometers should be operated at the lowest feasible concentration range.

For our instrument we use the following dilutions:

0.3 cc. to 100 cc. for urine sodium determinations;
0.2 cc. to 100 cc. for urine potassium determinations;
0.1 cc. to 100 cc. for serum sodium determinations;
1.0 cc. to 100 cc. for serum potassium determinations.

Advantages

For a laboratory where many sodium and potassium determinations are made the flame photometer can save a considerable amount of time because no ashing or other preliminary preparation of the sample is necessary. It is sufficient to merely add lithium to the unknown and make the proper dilution.

Another advantage is the small amount of serum that is necessary for the determination.

Summary

A brief description of the mechanism and operation of the Barclay Flame Photometer has been given, together with data from some tests run in this laboratory.

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MICRO-METHOD FOR THE DETERMINATION OF NON-PROTEIN NITROGEN*

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The trend at the present time is toward the use of less blood, time, and reagents in the determination of levels of the various chemical constituents of blood. Here at Saint Anthony Hospital we have been fortunate in obtaining many of our micro-chemical methods from Dr. Natelson of Rockford Memorial Hospital. This work was primarily started for a study of base-line chemical values in premature infants in our nursery. Until recently blood urea nitrogen[1] and glucose were the only Folin-Wu filtrate tests done by micro method. The non-protein nitrogen still required a vein puncture and a rather long time to digest the final filtrate. After a long period of trying different dilutions and comparing our results with the standard macro method we have developed a test which is within 0.4 mg.% of the macro test. This proves out consistently in spot checks of our regular N.P.N. tests as performed on our patients.

With a few exceptions this test follows the regular macro method determination but consumes much less time, reagents, and expensive glassware.

Expensive N.P.N. tubes are eliminated as our test requires smaller tubes which we mark at the proper dilution with an engraving tool used to mark surgical instruments. The tubes used for this purpose are regular pyrex tubes measuring 18 x 150 mm. and marked at 7 cc. and 10 cc. These marks are one-fifth the markings on regular N.P.N. tubes.

* Read before A.S.M.T. Convention, Portland, Ore., June 1952.

Procedure

1. Place 3.9 cc. of the precipitation reagent in a regular Wassermann tube 13 x 100 mm. By finger puncture obtain 0.1 cc. of blood using a micro pipet graduated at 0.1 and 0.2 cc. Blood must be measured accurately. As a puncturing instrument we find the No. 11 Bard-Parker blade through a cork gives the best free bleeding wound. The 0.1 cc. of blood is added to the 3.9 cc. of precipitation reagent. (1-40 dilution.) The pipet is rinsed in the solution until all traces of blood have been removed from it. The solution and blood is mixed by inversion. After the blood precipitate has assumed the chocolate brown color, it is centrifuged for about 3 minutes.

2. Two cc. of the supernatant fluid are removed with a volumetric pipet and placed in the special N.P.N. tubes previously described (graduated at 7 cc. and 10 cc.). Two glass beads are added as in regular N.P.N.

3. Add 0.2 cc. of the acid digestion mixture, which is the same mixture used in the regular N.P.N. determination.

4. The mixture is then digested over a micro burner. Since we are using lesser amounts of all constituents, less time is consumed in the boiling off of the water and since the dilution of blood is such (1-40) that there is very little material to be digested, care must be exercised so as not to carry digestion too far with the resulting cloudy N.P.N. After boiling the mixture to the point where it assumes the oily consistency of the acid and begins to boil more slowly, the watch glass cover is placed on the tube. The mixture will then turn a dark brown color but not as black as the regular N.P.N. because there is less material to digest. Continue heating very cautiously at this point as the solution will turn very rapidly back to a light color. As the fumes come off and the solution begins to assume a greenish clear consistency, stop heating. The tube being hot will carry digestion a little further. Regulation at this point will be much easier after 5 to 10 tests.

5. Allow the mixture to cool now for about ½ minute; then add 3 to 4 cc. of distilled water and let cool to room temperature. Cooling now takes less time as the amount of solution and the size of the tube is considerably less.

6. After the test has cooled to room temperature, add distilled water to the 7 cc. mark and Nesslerize to the 10 cc. mark.

7. For the standard we use ½ cc. of our regular N.P.N. standard in our special tubes (7 cc. x 10 cc.). Add 0.2 cc. of digestion mixture and dilute to the 7 cc. mark with distilled water and to the 10 cc. mark with Nessler's reagent.

8. Read in colorimeter and use the formula:

$$\frac{\text{Standard}}{\text{Unknown}} \times 0.15 \times \frac{40}{2} \times 100 = \text{mgm.\%}$$

NOTE: We find that the use of a filtrate of 1-40 dilution gives best results using 2 cc. of this. We tried 1 cc. of 1-20 dilution but found the end-point of digestion harder to control, with resulting cloudy mixtures. Also, the higher concentration of the solution makes the 0.2 cc. collecting pipet harder to clean and this could influence the accuracy of blood measurements and likewise the final result.

Reagents Required

Precipitating Reagent:[2] 20 cc. of 2/3 N. sulfuric acid is placed in a 500 cc. volumetric flask; add 20 cc. of 10% sodium tungstate and dilute to the mark.

Standard Solution:[3] Dissolve 141 mg. C. P. dry ammonium sulfate in distilled water and dilute to exactly one liter in a volumetric flask. 5 ml. = 0.15 mg. N.

Acid-digestion Mixture: Mix 300 ml. of 85% phosphoric acid with 100 ml. of concentrated sulfuric acid. Add 50 ml. of 5% copper sulfate and then 450 ml. of distilled water.

Nessler's Solution: Prepared according to the directions accompanying the commercial salt.

Conclusion

The test allows the use of a small amount of blood such as can be obtained from a finger puncture. This is especially useful with infants and small children. Reagents used for the test are used in 1/5 quantities as compared with the regular N.P.N. The time needed to digest and boil off excess water is greatly reduced (1½ min.) as compared to 3 to 4 minutes for the macro method. Time needed for cooling the solution is reduced considerably because of the smaller amount of solution to cool and the smaller diameter of the tubes used. The cost of glassware is reduced as the larger pyrex N.P.N. tubes are eliminated in favor of the small pyrex test tubes.

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A SPECTROPHOTOMETRIC MODIFICATION OF THE CEPHALIN-CHOLESTEROL FLOCCULATION TEST*

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The Hanger cephalin-cholesterol flocculation test has been accepted as one of the standard tests for the diagnosis and prognosis of diseases involving parenchymal liver damage. We have found the method currently employed is subject to at least two sources of error: (1) the amount of cephalin and cholesterol present was found to vary in the antigen suspension, and (2) the visual method for reading the test does not lend itself to a quantitative procedure. The purpose of this study was to improve the quantitative aspects of the test, thereby rendering it more useful clinically

Materials and Method

Preparation of serum: Blood was collected aseptically, and serum was prepared in the usual manner. Exposure of blood and serum to direct sunlight was kept at a minimum to prevent false positive reactions.[1,2]

Preparation of antigen suspension: The cephalin-cholesterol antigen produced by Wilson Laboratories was employed exclusively in this study. Consistent results were not obtained with other commercial preparations. Five ml. of ethyl ether were added to one unit of commercial antigen and one ml. of this stock solution was added dropwise to 35 ml. of distilled water at 67 ± 1° C. The suspension was boiled to a volume of 30 ml., cooled to room temperature and then adjusted to 35% transmittance by the addition of distilled water, if necessary. The transmittance was determined in 12 x 75 mm cuvettes against a distilled water blank in a Coleman Junior Spectrophotometer at a wave length of 580 mu.

Performance of the Test: Duplicate tests were performed in 15 ml. graduated centrifuge tubes employing the method described by Hanger.[3] A serum control containing 0.2 ml. of the test serum and 5 ml. of 0.85 per cent saline was prepared for each test. An antigen control containing one ml. of antigen suspension and 4.2 ml. of saline was included with each group of tests.

The tests were allowed to stand at room temperature in the dark for 24 hours. The tubes were then centrifuged for 15 minutes at 1900 rpm and the supernatant liquid poured into 12 x 75 ml.

* Presented at Colorado S.S.M.T. Convention, April 1952.
The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Medical Department, U.S. Army.

cuvettes. The transmittance of the supernatant fluid from each of the tests was determined against its serum control blank at a wave length of 580 mu. The per cent transmittance of the antigen control was read against a distilled water blank. A standard curve was then prepared with the transmittance of the antigen control representing zero per cent flocculation and 100 per cent transmittance representing 100 per cent flocculation. (Figure 1) The per cent flocculation of the test could then be determined directly from this standard curve. This method of reporting the test was based upon the amount of antigen remaining in suspension and not upon the amount of flocculation present.

Results

The modified cephalin-cholesterol test was performed on 30 serum samples from patients with parenchymal liver damage and 150 patients with no apparent liver damage. Figure 2 presents the results obtained with serum samples from 15 patients representing all stages of clinical hepatitis. The vertical lines represent the range of visual readings made by six persons experienced in reading the cephalin-cholesterol test. The circled point represents the average of the six readings, and the square represents the per cent flocculation as determined by the spectrophotometric method. In general, the average of the visual readings correlated very well with the spectrophotometric readings.

The large variation in the visual readings of case number eight was due to a high icteric index. In some tests, an extremely fine flocculation caused false negative visual readings; however, the same tests revealed up to 50 per cent flocculation when determined spectrophotometrically.

A patient suffering from infectious hepatitis was tested at intervals during his convalescent period. Table I compares the results of the two methods. The spectrophotometric procedure showed a constant decrease in the amount of flocculation which indicated steady improvement in the patient's condition, whereas the visual readings showed no change until the eighth week.

TABLE I

Visual and Spectrophotometric Results of Cephalin-Cholesterol Flocculation Tests for a Three-Month Period on a Convalescing Hepatitis Patient

Time (Weeks)	Range of Visual Readings	Average of Visual Readings	Per Cent Flocculation Spectrophotometrically
0	2—4	3	80
2	2—4	3	75
4	2—4	3	60
8	0—1	1	15
12	0—1	±	0

Employing both methods, a series of tests was performed in duplicate, the first being read at 24 hours, the second at 48 hours. The results can be seen in Table II. Only 5 per cent of these cases showed a slight change after the first reading. A five per cent change in flocculation was not considered significant. From these results and other data it was decided to eliminate the 48 hour readings, thus cutting in half the time necessary for performing the test.

TABLE II

Amount of Change in Per Cent Flocculation and Visual Reading Between the 24 Hour and 48 Hour Tests in 110 Serums

No. of Cases	Change in Per Cent Flocculation	No. of Cases	Change in Visual Readings
68	0	80	0
36	5	27	±
6	10	3	1+

Discussion

Since the spectrophotometric method was based on the amount of antigen in the supernatant liquid after centrifugation it was necessary to standardize the antigen suspension. Variance was noted in the amount of cephalin-cholesterol per commercial unit and in the concentration of the ether stock solution due to evaporation upon successive use. Uniform particle size and concentration of the antigen suspension were achieved by rigid temperature control and by adjustment of the per cent transmittance to a constant value.

The effects of aging on the working suspension was also investigated using known normal and hepatitis serums. The hepatitis serums were included to insure flocculation with antigen suspensions under investigation. One day old suspension kept at room temperature in a cotton plugged container gave positive tests with normal serum, even though the antigen control remained stable for over fifteen days. When it was kept tightly stoppered at 4° C. no false positives occurred up to eleven days.

Early in this investigation, it was believed that the amount of precipitate after centrifugation might be used to determine the extent of liver damage. During this phase of the study 6.5 ml. McNaught graduated centrifuge tubes were employed. The range of values, however, proved to be too limited to be practical. This method might be feasible if a centrifuge tube with a capillary bore were employed.

Readings made by the conventional visual manner were not reliable and in some instances not reproducible. Results varied

when six technologists read the tests independently, Figure 2. Reading the tests with the spectrophotometer eliminated this variable and placed the test on a quantitative basis.

It was found that differences encountered both visually and spectrophotometrically in 24 and 48 hour readings were not significant, and therefore the latter readings were eliminated, allowing a shorter interval between request and report to the clinician. This test has been used with excellent results for the past year in this laboratory.

Summary

A modified method for performing the cephalin-cholesterol flocculation test is presented. The method eliminates the human error in reading the tests and has the added advantage of reporting results as per cent flocculation. It is the opinion of the authors that these modifications render the test more valuable for diagnosis and prognosis of diseases involving parenchymal liver damage.

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PRINCIPLES OF EXFOLIATIVE CYTOLOGY**

By WARREN C. HUNTER, M.D.*

Everyone who has examined body secretions and excretions such as sputum, urine, feces and fluids, such as pus or transudates derived from body cavities, has some familiarity with cells of tissue origin in exfoliated or desquamated form. For the most part, however, we are accustomed to think not so much of the types of cells, other than leukocytes, but of what bacterial organisms or parasites are present in the material being studied.

One may fairly ask whether it is possible to differentiate normal cells from neoplastic ones in desquamated form. It so happens that there are inaccessible locations from which biopsy material is either difficult to obtain or cannot be gotten at all. If, then, cancer cells can be distinguished from those normally present in any location, and especially from those organs where biopsies cannot be obtained, a method which will permit their recognition is worthwhile. Or, even in such locations as the uterus or the bronchi, from which biopsies can be taken in most instances, the study of exfoliated cells may be of considerable value. Experience has shown that cervical cancer in its early stages may be small and difficult for the gynecologist to see. Under this circumstance a biopsy, or even multiple biopsies, may miss the cancer. Neoplasms involving the bronchi may well be beyond the reach of the bronchoscope, leaving the study of aspirated mucus as the only means of securing cancer cells. It is a known fact that any cancer which reaches a free surface loses its cells at a greater rate than normal cells from the same location are shed, and just as regularly. It need scarcely be said that the earlier a cancer can be diagnosed, the greater is the hope of cure.

Is it possible to identify cancer cells and to distinguish them from normal cells as both lie free, singly or in small groups, in mucus, in pus, in fluids? The answer is yes, within certain limits.

What are the limits? Ordinarily, desquamated cells, normal or cancerous, preserve their identity and staining characteristics for a variable time. However, in hypertonic or hypotonic solutions, or if the area from which they come is badly infected or necrotic, all kinds of cells break down more rapidly than under normal circumstances. Cells, shed into mucus, seem to retain their form and to stain well for an appreciable time. Another limitation, and an important one, is governed by the skill and care of the person who obtains the material and makes the smears and by whether or not they are very promptly and adequately fixed. Very thin and very thick smears, just as with blood or when one

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is searching for bacteria, are unsatisfactory; the first, because of the dilution factor, the second, because one cannot see through thick areas. Folding or doubling over renders some cells unsuitable for recognition. Proper staining of smears is of the utmost importance and this is a prime responsibility of the medical technologist. The greatest drawback and danger of all lies in the attempt of poorly trained persons to interpret the characteristics of cells. Another limitation is that not every smear from a given location, even though known to be the seat of cancer, may contain cancer cells, or these may be so sparse that unless the examiner searches very thoroughly the cells may be missed.

Do cancer cells differ from normal cells, and if so, how? Although subject to variation in size and shape, normal cells have nuclei that are not unduly large, usually a large amount of cytoplasm per cell, and the nuclear chromatin is neither excessive nor abnormally distributed. Normal cells are more likely to come off singly than in groups. Cancer cells, on the other hand, show an increased nuclear-cytoplasmic ratio, vary more in size and shape, even among themselves, tend to desquamate in groups, often lack clear individual cell definition, and of the greatest importance, the nuclear chromatin is excessive enough so that one appreciates this factor (as well as increased size) in scanning the section or smear under the low power magnification. Furthermore, the chromatin lacks the regular or smooth distribution of a normal cell of the same kind; the chromatin is likely to be coarse, in clumps of varying size, with clear spaces between. Not infrequently one or more nucleoli are apparent. Actually, these same principles are employed in the recognition of immature blood cells as one studies peripheral blood or marrow, so that there is nothing new or mysterious about the matter. With adequate training in a laboratory staffed by experienced personnel and with a sufficient number of authenticated smears for study, it is just as possible for a medical technologist to become proficient in the finding and recognition of cancer cells as it is to unravel the difficult problems of differential blood or marrow counting. As in hematology, so in the application of exfoliative cytology to the diagnosis of cancer, the final responsibility for rendering the diagnosis lies with the physician. It is his duty, first of all to have special training in this field, and second, to study the marked cells of the smear.

It must be distinctly understood that exfoliative cytology is not intended to replace the biopsy. In fact, the cytologist sincerely wants the biopsy, if for nothing more than confirmation, in every case where it can be obtained. The method is an adjunct one but nevertheless in certain locations such as the uterus, a most valuable means of finding small and early cancers and pointing to the necessity of adequate biopsy immediately. In

other locations, the bronchi for instance, the aspirated material should always be studied, even though what appears to be an adequate piece of tissue has been gotten.

There is great need today for far more medical technologists to take special training in exfoliative cytology. The physician simply does not have the time to spend on the thorough and honest search for cancer cells. It is most helpful to have trained medical technologists do this part of the work and only when enough such persons are available will the method receive the wide use it should.

NEW USES OF THE MALE FROG TEST AND REVIEW OF METHODS*

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Methods of the male batrachian test are reviewed and suggestions for expansion of its uses are made. From our experience, we feel that this test is as reliable as and more rapid and economical than other tests for detecting the presence of chorionic gonadotropic hormone. One author is quoted as saying, "The presence of chorionic gonadotropic hormone in the urine of a male is an indication of chorionic tumor tissue. Its decrease means removal or regression of the tumor tissue; its increase may mean spread of the disease or may be the result of an increase of FSH (Follicle-Stimulating Hormone)."[1] Many urologists believe that it is necessary only to detect the presence of chorionic gonadotropic hormone; but a table is included which shows that the test may also be used for quantitative analysis.

Results of the male batrachian test were determined by reports the physicians gave us indicating success or failure with our technique. Mimeographed sheets were sent to individual doctors to report results, and hospital charts were also checked. From these data we were able to ascertain much valuable infor-

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mation: such as how early we could rely upon this test, whether tubal pregnancy would give a positive test, or if certain drugs interfered with the test. Our survey has shown positive tests on all cases of tubal pregnancy examined. Of especial interest were hydatiform mole cases where the titre remained for several weeks following the passing of the mole, and a case of chorio-epithelioma in which positive reports were obtained on .001 cc. urine, on .1 cc. blood plasma, and on 1 cc. of cerebrospinal fluid obtained at postmortem. All tests in this case were completed in a four-hour period, whereas Ascheim-Zondek tests would have taken at least five days. Postmortem examination revealed chorio-epithelioma involving the lungs, brain, left kidney, and small bowel. There was no primary tumor found in the uterus, cervix, vagina, tubes, or ovaries. The chorionic gonadotropic hormone manifested itself by thickened endometrium, multiple corpus luteum cysts in the ovaries, and active breast tissue, which were all seen microscopically.

Survey sheets for female patients numbered a total of:

True positive	229	(Pregnancy)
True positive	1	(Chorio-epithelioma)
True positive	6	(Hydatiform mole)

Thirteen tubal pregnancy cases are included in this group; ten reacted positive due to retained placental tissue. The cases of hydatiform mole and chorio-epithelioma were counted as true positives since chorionic epithilium in these instances secretes the hormone present in pregnancy.

False positive	1	(Ovarian cyst)
True negative	109	(Pregnancy)
False negative	1	(14-16 weeks' pregnancy with extensive pelvic malignancy)
False negative	1	(Too early for elevated titre of hormone)

Tests performed on male patients:

Positive	1	(Embryonal carcinoma of testicle)
Positive	1	(Chorio-epithelioma of testicle with extensive metastases)
Positive	2	(Teratocarcinoma)
Negative	1	(Teratoma removed 3 years previously at which time a Friedman test was negative. A malignant appearing lesion was found in the lung; lung surgery was performed, revealing scar tissue not of malignant origin.)

Since our survey we have made the observation that this test is excellent to determine whether placental tissue is retained, and the physicians use this in deciding if dilatation and curettage

are needed. If there is viable tissue, a positive test is obtained; if necrotic tissue is present, the test will likely be negative. This observation has been substantiated by checking the pathology reports.

The question arises as to how early a positive test can be obtained. This varies, as it is dependent upon the amount of hormone the patient excretes. The earliest has been seven days after the first missed menstrual period (21 days after conception). This patient was on a 28-day cycle. The average time for obtaining a positive test has been 20 to 30 days after the first missed menstrual period; however, we have no assurance but that a positive test could have been obtained earlier if the patient had presented herself to the physician earlier.

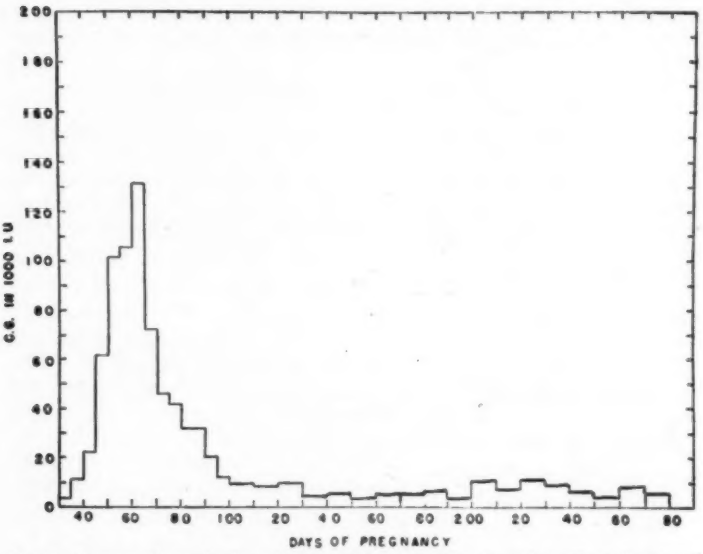

Fig. 1. Average Concentration of Serum Gonadotrophin at various times in normal pregnancy, based on 24 cases. Delfs, from Eastman, N. J. Williams Obstetrics, 10th Edition, 1950, Courtesy of Appleton-Century-Crofts, Inc.

Our one false positive test was obtained with a simple ovarian cyst.[2] Twelve tests were performed through a period of 16 weeks, and a positive result was obtained each time. The pathological report of the tissue removed surgically was serous cystadenoma of the ovary. The test became negative 24 hours after the cyst was removed. Unfortunately, none of the cystic fluid was obtained to check whether it would yield a positive test.

We have checked the test with various drugs to determine their ability to give positive reports. Purposely we have tried many urines of patients receiving ACTH and Cortisone, but none has given false positive reports. One patient who had myasthenia gravis and was taking prostigmine gave positive results of the test on each of six trials. An exploratory laparotomy was done and she was found to have a tubal pregnancy.

It has been claimed that patients with severe liver damage give false positive results.[3] In our trial checks, using urines from patients with severe liver damage, none has given false positive reports. A series of 14 hepatitis cases with liver damage gave no false positives; also three acute obstructive jaundice cases yielded no false positive results. This is possibly due to our adsorption concentration technique.

Much interest has been shown as to the use one could make of the test as a quantitative assay.

Comparison of Titre of Hormone on the Same Urines

	Male Frog Test (Positive)	Aschoim-Zondek Test
Chorio-epithelioma	0.001 cc. urine 0.1 cc. blood plasma 1.0 cc. spinal fluid	Positive on 1-100,000,000 Negative on 1-1,000,000,000
Hydatiform mole with ovarian involvement	No Test 0.05 cc. urine 1.0 cc. urine 5.0 cc. urine 10.0 cc. urine	Positive on 1-10,000 No test Positive on 0.1 cc.; negative on .01 cc. Positive on 1.0 cc.; negative on 0.1 cc. Positive on 1.0 cc.; negative on 0.1 cc.
Embryonal carcinoma, testicle	0.5 cc. urine No reduction after surgey	Positive on 1-10,000 Negative on 1-100,000
Teratocarinoma, testis	1.0 cc. urine No reduction after surgery and x-ray	No test
Chorio-epithelioma of testicle	0.1 cc. urine	No test
Teratocarcinoma, testis	0.01 cc. urine	Positive on .01 cc.: negative on .001 cc.

In surveying the literature one notices that the male batrachian test is used in all continents of the world and on many of the larger islands. Many methods are employed and many different native species are used; however, in the adoption of this test we have had to work with amphibia that could be obtained in our locality and we felt it was necessary to establish a strict regime for its use. In our hospitals we selected the Rana pipiens frog for use from September until June; then during June, July, and August the Rana clamitans is used. The reason for this choice is because the Rana pipiens is an earlier breeder than Rana clamitans. We have tried other species but these were the most available commercially for use. Care of the animals is extremely important. To perform the best work and to obtain good results,

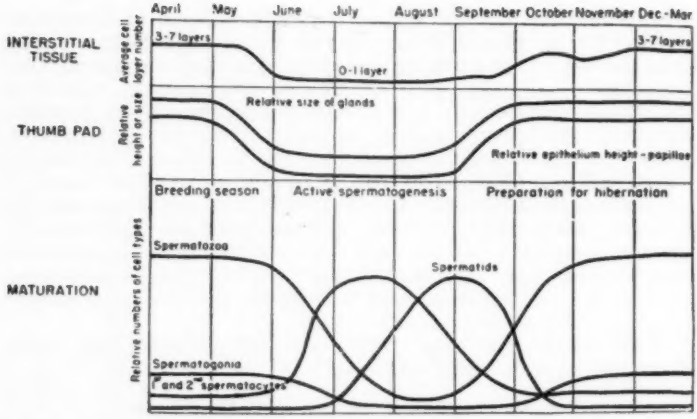

Figure 2. Normal cyclic changes in the primary and secondary sexual characters of the frog, Rana pipiens. (Courtesy of The Blakiston Co.)

it is very necessary for one to know the habitat, anatomy, and the physiology of the animal used for experiments.

Various investigators have tried to evaluate the sensitivity of the animal by checking with standard amounts of chorionic gonadotropin preparations. One of the first investigators to use this method was Hartmann-Perdomo who reported that 9.6 International Units were the stimulating dose for Rana pipiens.[4] Other investigators claimed different doses were necessary; some gave lower values, other higher.[5,6,7] This variance may be due to climate or locality. This meant that we must check each lot of animals received. To date our lowest number of International Units for response has been 10 I.U. by intraperitoneal injection. (The International Unit is defined as the gonadotropic activity of 0.1 mg. of a standard preparation of chorionic gonadotropin. This unit was adopted in 1938 (League of Nations Health Organization Bulletin).)

Method

Preparation of Urine Specimens:

1. Place 100 cc. of fresh urine, preferably from the first morning voiding and with a specific gravity of at least 1.010, in a 250-cc. Erlenmeyer flask.
2. Using nitrazine paper (E. R. Squibb and Sons), check the pH of the specimen.
3. Adjust the pH to 4.5 with 20% hydrochloric acid, using a fine-tipped pipette for the addition of the acid.
4. Add 5 cc. of 20% washed kaolin suspension to the urine sam-

ple and mix thoroughly by inverting the flask or by swirling. Be sure to mix the kaolin suspension well before using.

5. Allow the mixture to stand for at least an hour, not more than one and one-half hour.
6. Transfer the mixture to several large tubes and centrifuge until the kaolin is packed in the bottom of the tubes. The hormone will now be found adsorbed on the kaolin.[8]
7. Discard the supernatant and allow the tubes to drain. Wash the kaolin with distilled water to remove acid; then centrifuge again and pour off the supernatant fluid.
8. Add 5 cc. of 0.1/N sodium hydroxide to one of the tubes containing the kaolin sediment and shake well to insure good mixing, preferably 5 minutes, allowing the dilute alkali to elute the hormone. Transfer the contents to the next tube and mix, so that the same alkali will be used to elute the hormone from the entire volume of kaolin used to collect the hormone originally. Add one drop of phenolphthalein to see if alkaline; if not, add more sodium hydroxide. It *must be alkaline* to elute the hormone.
9. The mixture is recentrifuged after standing 15 to 20 minutes. The supernatant will be red; add enough 20% hydrochloric acid to bring about a pH of 6.5 (Nitrazine paper check).
10. Collect the supernatant which should be clear.
11. If the solution is colorless but markedly turbid, recentrifuge to remove the finest particles of kaolin.
12. Transfer the solution to a 5 or 10-cc. syringe fitted with a 25-gauge needle. The solution is now ready for injection.

NOTE: The above preparation is based on 100-cc. amounts. If the only urine obtained has a specific gravity of 1.005, give 4 cc. of concentrate; or, if only a 50-cc. amount is brought in, give 4 cc. of concentrate.

Preparation of Frogs for Injection:

1. Remove the frogs from refrigerated storage and place each one in separate numbered containers in which there is one-half inch of cold tap water, the temperature of which should not exceed 20° C. so that the frog is kept as near his normal environment as is possible. (Convenient containers are pint-sized canning jars having covers fitted with wire mesh or perforated to admit air. The water bath can be kept at the desired temperature by placing the jars in a pan into which cold tap water is run or a small amount of ice placed).
2. After the frogs have warmed up, remove them one at a time from their jars and examine the urine of each for spermatozoa as follows:
3. With a soft towel, gently remove excess water from the lower

body and from the hind legs. Pull the rear legs towards the body and gently press the thumb against the animal's abdomen.

4. Place a 3″ x 1″ microscope slide against the anal opening and collect a drop or two of urine.
5. Using the high dry objective for a **final** check, examine each slide for the cigar-shaped spermatozoa. With proper light adjustment, it may be possible to see flagella. The spermatozoa are often found clustered around shed Sertoli cells (blast or nurse cells of the sperms). If spermatozoa are found in the frog urine **before** injection, the animal should not be used for this test. As a rule, spermatozoa will not be found before injection if the frogs were inactive during the summer months.

Fig. 3. Proper position to hold frog when injecting dorsal lymph sac.

Injection of frogs with hormone concentrate
(for routine pregnancy test):

1. Injections are made into the dorsal lymph sac. Hold the frog firmly in one hand, clasping the midline of the animal between the index and the next finger. Use the thumb to hold the animal. The frog's head should point toward the heel of the palm.
2. Directing the needle posteriorly, enter the skin just above the junction of the hind legs and body.
3. Inject 2 cc. of the hormone concentrate.
4. After injection return the animal to the proper jar, adding enough alkaline or neutral water to make a layer of about one-half inch in the bottom of the jar.

NOTE: When performing tests on urine where teratoma or chorio-epithelioma might be present:

Use 5 cc. urine and inject into the dorsal lymph sac. If positive results are obtained, use 1 cc. If the test is positive with the use of 1 cc., then .1 cc. is used. It is best to then use serial dilutions in order that the amount injected will not be less than 1 cc. Always make dilutions with a volumetric pipette. When injections of only 1 cc. are given, use a tuberculin syringe. When dilute injections of 1 cc. are used, the intraperitoneal route is to be preferred because of the greater amount of absorption space.[5] There is also less leakage than from the dorsal lymph sac.

Reading the Test:

1. Wait 20 to 30 minutes after injection before attempting to read the test. At the end of this period, remove each animal from its container, collect and examine the urine precisely as described under **Preparation of Frogs for Injection.**
2. If no spermatozoa are found at this time, return the animal to the jar and wait for about one hour; then repeat the examination of the urine. If spermatozoa are present, the test is positive. If only 2 to 4 spermatozoa per high power field are found, the test is repeated on another specimen. We prefer to see many sperms in the field migrating around the Sertoli cells. If parasites, especially protozoa, infest the animal, one must make sure he can see the sperms and that he is not viewing the flagella of the parasites.
3. If at the end of 4 hours following injection the urine is still devoid of spermatozoa, inject 2 cc. of known positive concentrate and again check the urine for spermatozoa. This procedure is necessary to exclude the possible use of a sterile animal. While this is not usual, it may be likely during the months of June, July, and August.
4. Report the results of the test as "MALE FROG TEST POSITIVE" or "MALE FROG TEST NEGATIVE." This is to avoid confusion with other tests in which female frogs are used.

Notes on Frog Care

1. When animals arrive from the biological supply house, they should be unpacked immediately. During the unpacking process each animal is examined for sex to make sure only male animals are used. Males may be distinguished by the larger thumbs, whereas in the female frogs the digits are all the same size.

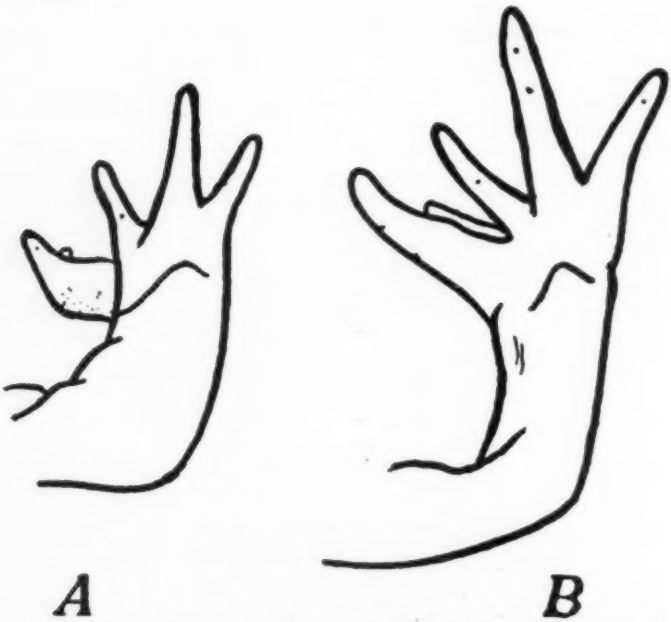

Fig. 4. A. Hand and forearm of male frog. Note thick thumb pad and mottled skin. B. Forearm of female frog. Note digits almost same size, clear skin.

Also careful investigation is made to notice if there is any evidence of "red leg." This is a disease caused by Bacillus hydrophilus fuscus, an organism carried in the gall duct of the frog and excreted in the feces. We have found that "red leg" may be controlled by the use of the preparation Chloromycetin Palmitate (Parke, Davis and Company), an oral suspension of chloromycetin in which each teaspoon represents 125 mg. chloromycetin. It is given in proportions of 10 cc. of Chloromycetin Palmitate diluted to 100 cc. with distilled water and approximately 1 cc. administered orally to each frog before refrigeration. Oral administration is easily

accomplished by using a 20-cc. syringe fitted with a long blunt needle such as that used for filling sedimentation tubes or a blunted lumbar puncture needle. As each animal is removed from the shipping box, 1 cc. of chloromycetin is given orally. The antibiotic properties of this preparation are not active until the suspension is given orally. This ester of chloromycetin has no microbiological activity until it is hydrolyzed by lipase in the gastro-intestinal tract, releasing chloromycetin. The lipase is secreted by the pancreas of the frog.

2. Animals are refrigerated at 4° to 6° C. in aluminum pans (junior broilers) measuring 11.5 x 7.5 inches fitted with aluminum covers having perforations in the lid. Not more than 12 to 15 animals are stored in each pan. Alkaline or neutral tap water is kept in the pans to the depth of one-half inch. The frogs are rinsed each 48 hours by merely pulling back the lid and allowing cold tap water to rinse out the excreta. Cold tap water is again added to a depth of approximately one-half inch. Any animals that might be victims of "red leg" are removed. We have found it best to keep them for only about one month in refrigeration. Our need is heavy so we usually order each two weeks. If one finds a good biological supply house, it is best to make a contract.
3. Frogs kept at this temperature are in a state of hibernation nearly like their state during the winter months. **They need not be fed.**
4. When animals are received from the biological supply house, it is best not to use them for 2 to 3 days. This is especially true in spring months when spermatozoa might be found in urine. All checks with known amounts of chorionic gonadotropic solution should be made after placing the animals in the refrigerator for 2 to 3 days.
5. Rana pipiens are not quite as large an animal as the Rana clamitans we use during the months of June, July, and August; however, we prefer the "jumbo size" of Rana pipiens weighing 40 to 50 grams.
6. At present we are checking each lot of frogs received with known amounts of International Units of gonadotropic powder. If we find the animals are not too sentitive, we give more urine concentrate. The 2-cc. amount of urine concentrate used in the routine pregnancy test is based on sensitivity to 10 I.U.
7. In standardizing each lot of animals, we purchase standard chorionic gonadotropic powder and prepare dilutions in sterile vaccine bottles. It is best to make dilutions to have 1 cc. of diluted material containing 10 I.U. (Products used have been Antuitrin "S," Parke, Davis and Company; and Follutein E. R. Squibb and Sons).

8. It is extremely necessary in working with the chorionic gonadotropin solution to use sterile tuberculin syringes for accurate measurement.
9. Another method to standardize and check animals when negative reactions occur is to save urine from a known pregnancy on which an Ascheim-Zondek or previous male frog test has been performed. If units were expressed, and 1 cc. would give a positive reaction, freeze the urine in small tubes in 1-cc. amounts; then when negative tests occur, the animal is checked to see if it is a non-reactor, and after a period of 4 hours it is checked with the known positive.
10. We wish to emphasize that animals **must always be checked.** Some claim to dissect the testicular tissue from the frog. We find it easier to check it physiologically for its function; then the animals can be used by other departments for experimental or class work.
11. We have found that freezing does not seem to have any effect on the sensitivity of the hormone. Urine from a patient with chorio-epithelioma, hydatiform mole, or a hormone-producing testicular tumor is frozen in the lowest reacting amounts. For example, if .1 cc. gave a positive reaction, add .9 cc. of normal saline and freeze.

Conclusions

1. The male frog test is applicable for quantitative analyses in cases which formerly required longer and more expensive tests.
2. With proper animal care and the use of healthy, standardized animals, the male frog test is an accurate, rapid, and economical one to use; therefore, the physician can depend upon it for emergency cases.
3. It is extremely important that the individual performing the **test must have knowledge of the care and physiology of the frog.**
4. Breeding habits of different species of frogs have to be taken into consideration in the selection of species for use in this test.
5. Animals need not be sacrificed with this test and can be used by other departments for experimental purposes or for class work.
6. Accurate results and techniques are more assured as the low cost of the animals permits the use of several for one examination, and the time delay is a matter of hours rather than days.

SHEET FOR TABULATION OF DATA ON MALE FROG TEST

Doctor's Name_____ Patient's Name_____

Hospital_____ Hospital Number_____

Brief history, which includes why test is being performed or patient was admitted to the hospital. Also state if a sterility problem is encountered.

Any history of abortion_____

Amount of time since last period was missed_____

Medications including ordinary drugs and hormone therapy. State specific drugs and hormone given, amount and when last dose given.

Results of other tests for pregnancy.

If surgery is performed, surgical findings.

Results and follow-up of patient as to facts.

False positive_____ False Negative_____

True positve_____ True Negative_____

Estimate number of spermatozoa per H.P.F._____

Urine Sample:

Sp. gravity_____ Sugar_____

pH when received_____ Albumin_____

Age (of sample)_____ Acetone_____

Species of frog used_____

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MacNEAL'S STAIN FOR BLOOD FILMS*

ANNE ADWAN, B.S., M.T. (ASCP)
Mercy Hospital, Oklahoma City, Okla.

Since Leishmann in 1901 modified Jenner's blood stain by substituting for Methylene Blue, a Methylene Blue which had been partly decomposed by treatment with dilute alkali and heat, there have been many imitations and slight modifications of the stain described by various workers. In this country the best known of these modifications is the one of Wright's. The results obtained in actual staining by the use of these various stains are as a rule inferior to the pictures obtained by the original stain of Leishmann.

Later in 1905 it was shown that there were four essential dyes in the Romanowsky type of stain of which Leishmann is a modified version. Geimsa maintained there were only three. These four dyes are Methylene Blue, Eosin, Methylene Azure and Methylene Violet. Satisfactory staining was done by using either M. Azure or M. Violet with the other two stains. However, the most brilliant and satisfactory results were obtained when all four dyes were used.

In 1913 Doctor W. J. MacNeal in collaboration with Schule came out with the following formula which gave excellent results in staining blood and blood parasites.

1 gm. water soluble Eosin
2 gm. Medicinally pure Methylene Blue
0.2 gm. Methylene Violet (Bernthen's)
0.6 gm. Methylene Azure (Azure A)

This is the formula we are now using in our laboratory and it is very easy to prepare and very economical. There is a Tetrachrome stain on the market which comes already mixed as a dry powder ready to dissolve in the proportions of 3 gms. of dye to 1 liter of Methyl alcohol. We have found these prepared stains unsatisfactory and prefer to make up our own.

The staining properties for MacNeal's stain are of course the same as those of Wright's. The white cells are very sharply stained, basophilic granules are especially well marked in all the cells and the nuclei are beautifully stained. If you do bone marrow smears you will be especially pleased with the staining qualities of MacNeal's stain. We feel that you get the very best results with the least effort with this stain. The timing is relatively unimportant as far as quality of the stain goes and we have found that the use of any buffer other than distilled water is completely unnecessary. As a matter of fact we tried buffers

* Read before ASMT Convention, April, 1952.

of various pH and found that distilled water was much better.

Unfortunately, we have never been called upon to diagnose malaria since we have been using this stain in our laboratory and cannot tell first-hand, so to speak, but can rely on the literature. The authors do feel that excellent results are obtained in staining parasites.

Conclusion

In conclusion, I would like to briefly go over the preparation of the stain as we use it. All four dyes are dissolved in a liter of freshly opened, pure, acetone-free Methyl alcohol. The solution is placed in a water bath at 50° C for 30 minutes and agitated frequently. Then it is placed in a 37° C incubator or oven for 3 to 4 days. The solution is then filtered just prior to use.

At times we have encountered one difficulty with the stain, that being precipitation of some stain on the smears. We have overcome this by flooding the stained smear with a little more stain just before washing, then a thorough washing with distilled water. This removes any precipitation which may be on the slide.

All in all we feel that the advantages in ease and economy of preparation, the uniformity of the stain and the fine characteristics of the prepared smears make it worthwhile to use MacNeal's stain for blood smears.

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2. Gradwohl, R. B. H., Clinical Laboratory Methods and Diagnosis, 2: 1317, 1948.

TRIBUTE TO DR. FLORENE C. KELLY

Dr. Florene C. Kelly is Acting Chairman of the Department of Bacteriology, The University of Oklahoma Medical School, having replaced Dr. Homer Marsh who is now Associate Dean of Students at the University of Miami School of Medicine, Miami, Florida. At present, Dr. Marsh is Acting Dean of the new school.

Dr. Kelly was born in Marion, Connecticut. In 1926, she received a B.S. degree from Simmons College, Boston, Massachusetts. She received an M.S. in 1936 and a Ph.D. in 1943, both from the University of Chicago. In 1944 Dr. Kelly was appointed Assistant Professor, Department of Bacteriology, The University of Oklahoma Medical School.

In collaboration with K. Eileen Hite, Ph.D., M.D., formerly of the Department of Bacteriology and Parasitology, at The University of Chicago, Dr. Kelly has published **MICROBIOLOGY**, 1949, which is reviewed in this issue of the Journal. She also has written several papers which have appeared in scientific periodicals.

Dr. Kelly is an honorary member of the Oklahoma County Society of Medical Technologists and the Oklahoma Society of Medical Technologists. She has contributed directly and materially to these organizations through arranging and conducting programs and through assistance to individual technologists in preparing original work, as well as being the bacteriologist to whom seemingly unsolvable problems are referred. Through these contributions, she has been of direct and indirect aid to the entire profession.

THE GAVEL

In this issue of the American Journal of Medical Technology you will see three announcements which demand your attention. The first is the announcement of the Nominations and Elections Committee; the second is the announcement of the Constitution and By-Laws Committee; the third is the announcement of the 1953 ASMT Program Committee. Let us discuss these three announcements for a moment.

Nominations and Eiections: This Committee is dependent upon you for the slate of officers and board members that will be finally selected. Begin now to think of members in your own state or in other states who would make a good officer or a good board member. Contact those whom you would like to suggest for the various positions that will be vacant, ask their permission to be suggested for the office, and ask each for a list of qualifications for office. As soon as you get these, send them to the Chairman of the Nominations and Elections Committee. Be sure to send six copies —one for each member of the Committee. This makes the work of the Committee easier. Remember—qualifications of your candidates, plus permission to be nominated for office must be in the hands of the Committee Chairman by December 1. This is your chance to nominate the people of your choice and have them qualified by the Committee.

Constitution and By-Laws: This Committee is also dependent upon you. What would you like to see incorporated into the Constitution and By-Laws? What would you like to see cut out? What wording should be changed in order to make the meaning clearer? This is your society. Its Constitution and By-Laws should be written by you. If you don't like any part of it, this is your chance to change it. If any part of it needs strengthening, this is your chance to do it. Send your suggested changes to the Chairman of the Constitution and By-Laws Committee, six copies, please, by January 1, 1953.

1953 ASMT Program Committee: I do not have to remind you about the important role this Committee plays in the success of any convention. This group has been asking you for contributions in the form of scientific papers for the 1953 convention since July of 1952. Don't you think it is about time that you let the Chairman of this Committee know that you are hard at work on a paper for the 1953 convention? We would like to see more of our members participate in the presentation of scientific papers. How about it?

Now this brings me to one more matter I want to mention to you—the News Letter. Most of you know that we are using the News Letter as the means of dispensing information to our members. Much of the material included in the News Letter must be repetitious because of the turnover in state officers and committee chairmen. Each new slate, whether officers or committee members, has to be told the same things that were told to their predecessors, and which for some reason was not passed on to the newly elected. So, those of you who get tired of seeing this information repeated can just turn to another page if you don't want to read it, but it is the best way we have of getting the information out to all who need it. Perhaps you are not State Treasurer, Membership Chairman, Public Relations or Recruitment Chairman. You could be, and if you read this material every time you see it, you will make a much better Chairman or State Treasurer. So there is an advantage in having the information in the News Letter—it not only reaches those who are directly concerned, but those who might be concerned in the future.

Let each of us resolve to criticize less and work harder for the constructive things. If Nominations and Elections doesn't present the slate YOU sent in, REMEMBER there are forty-eight other affiliate societies. If Constitution and By-Laws doesn't present what you thought should be included, perhaps YOU forgot to send in those amendments you were thinking about. If the 1953 ASMT Program isn't what you thought it

should be, perhaps you failed to suggest a speaker or to present that paper you could have presented. If the News Letter is larger than it was when it was first printed, it is because more and more the committees are using this as a medium for dispensing information to the states.

While you are thinking about constructive things for ASMT, send me items that you would like to have on the Agenda for the 1953 Advisory Council meeting.

Sadie Cartwright

KENTUCKY WELCOMES A.S.M.T. IN 1953

May we of the Bluegrass State offer you our southern hospitality, our beautiful Kentucky scenery filled with historic lore and recreational facilities, and our reputation for being the home of bourbon, beautiful women and fast horses. Kentucky hopes that many of you will find it possible to visit us next June for we feel that there is much of beauty and interest to enjoy in Louisville and its surrounding territory.

Within the city of Louisville itself there are many places you will want to see. Let me mention just a few: Churchill Downs, scene of the Kentucky Derby; the University of Louisville, established in 1798; Cherokee Park, one of the most beautiful natural parks in the nation which contains the oldest municipal golf course in the United States; Cave Hill Cemetery, a spot of outstanding beauty, in which the monument and grave of George Rogers Clark are located; the Cathedral of the Assumption, which is celebrating its centennial this year; the Courier-Journal and Louisville Times Building, a fine example of modern newspaper architectural design; Rubbertown, an industrial section of note; several large distilleries and, of course, the Beautiful Ohio spanned by the Clark Memorial Bridge, known as the Gateway to the South.

We sincerely hope that many of you will plan to stay long enough to realize the fact that Kentucky has a combination of tour attractions, including recreation, beautiful scenery and historic lore that can be enjoyed at a minimum expense. To suggest a few there are Fort Knox, the United States Gold Repository, Lincoln Memorial at Hodgenville, Mammoth Cave National Park, Cumberland Falls and Lake Cumberland, Kentucky Dam Village State Park with its new Kenlake Hotel, Natural Bridge in Cumberland National Forest, Fort Harrod at Harrodsburg, the oldest permanent settlement in interior Kentucky, Lexington, home of the University of Kentucky and of some of the most noted horse farms in the world, Frankfort, capital of Kentucky, Bardstown, one of the oldest towns in the state, where Stephen Collins Foster wrote his immortal song while visiting the now State Shrine, "My Old Kentucky Home," and St. Joseph's Proto Cathedral, the first Cathedral established west of the Alleghenies in which hang nine fine paintings by old masters, gift of Louis Phillippe, Duke of Orleans and later King of France.

With all this inducement you all will surely accept our warm and sincere invitation to come and visit with us in Louisville. Rest assured that we will do our best to make your stay one of utmost interest and enjoyment as well as one of beautiful and lasting memories.

Let us know where you prefer to tour in Kentucky!

We will be seeing you—YES?

Convention seals are being prepared! Write for information!

Ramona Barret Shaw,
Publicity Chairman
1629 Jaeger Ave., Louisville, Kentucky

COMMITTEE REPORTS

FINANCE COMMITTEE

To date the following requests have been received and approved by this Committee:

1. Legislative Committee, $300.00;
2. Membership Committee, $350.00;
3. Education Committee, $100.00;

At the present time two additional requests are being considered:

1. Standard and Studies Committee, $75.00;
2. Recruitment and Vocational Guidance Committee, $259.42.

Mrs. Ida C. Blorem, M.T. (ASCP), Lincoln, Nebraska, has been appointed to fill the vacancy on this Committee created by the death of Mr. Ralph Miller.

Mrs. Kathryn F. Dean, M.T., (ASCP), Chairman

CONSTITUTION AND BY-LAWS COMMITTEE

Suggested changes in the Constitution and By-Laws of the American Society of Medical Technologists must be in the hands of the Constitution and By-Laws Committee **not later than December 31st.** A suggested change should be accompanied by the reasons for the change.

Send six copies of your recommendations, one for each of the members of the Committee, to Rose M. Hachman, Chairman, 4200 East 9th Ave., Denver, Colorado.

RECRUITMENT AND VOCATIONAL GUIDANCE COMMITTEE

The National Recruitment and Vocational Guidance Committee of the ASMT has reviewed all types of recruitment programs for the State Chairman in the form of a work sheet. In addition to the outlining of various fields of action, members of the committee, both past and present, contributed helpful suggestions which they learned "by doing" in their recruitment work.

The Registry furnished a list of material and various papers, films, and slides on recruitment by Society members were collected and made available through the Executive office. One particularly new and worthwhile source of material to stimulate interest in the profession of Medical Technology is to be found in the seven sets of colored kodachrome slides recently edited and catalogued by Dr. Arbogast at the University of Indiana. In these slides the profession is depicted in a real and practical manner showing actual personnel performing ordinary laboratory procedures. Each set consists of over forty views and any one of the pictures may have been taken in your own laboratory. The Recruitment Committee highly recommends these slides and urges every State Society to include them in several of their programs throughout the year. These sets are available through the Executive office.

The State Chairmen were mailed an individual letter at the time the News Letter carried the Recruitment outline. They were encouraged to use the methods presented and to start organizing their program as early as September. Moreover, it was emphasized that if anyone felt that he was already over-worked, that would be all the more reason for realizing the necessity of a complete, successful and immediate recruitment program and acting upon that realization.

The Committee Members received a condensed report concerning the response of the individual states to last year's recruitment. The State Socie-

ties who did participate have been asked to share their methods and materials; those who did not respond are being encouraged to cooperate this year. Each National Recruitment Committee member has assumed her responsibility in the program and this is being accomplished by each member heading a group of states as her particular charge in seeing that the execution of recruitment programs is being organized and carried out by the State Chairmen.

Sr. Barbara Clare, M.T. (ASCP), Chairman

SCIENTIFIC EXHIBITS COMMITTEE

The Scientific Exhibits Committee reminds you that it is not too early to plan your 1953 ASMT exhibit in Louisville. Space is available at this early date, so now is the time to insure a place for your exhibit.

State societies, hospital groups, educational institutions, and individuals are urged to exhibit anything of a strictly scientific nature that would be of interest to Medical Technologists.

O. M. Alton, M.T. (ASCP), Chairman
Scientific Exhibits Committee
Norton Infirmary Laboratory
Louisville 3, Kentucky

NOMINATIONS AND ELECTIONS COMMITTEE

The Nominations and Elections Committee wishes to call to your attention the list of officers to be elected at the National Convention of the House of Delegates of the American Society of Medical Technologists in Louisville, Ky., in 1953. The committee has published announcements in the Newsletter and the Journal, requesting **all** members to send in their selection of candidates for the offices vacant to your committee. It is important that we have the names and qualifications of the proposed candidates from those who best know their qualifications. They must be members of ASMT in good standing and must have been members for at least two years. Now is the time for you to say whom you would like to have nominated to these positions. The task of picking candidates from all of those sent in is not an easy one and we hope you will have the faith in this committee that is due it.

ALL SUGGESTIONS MUST BE IN THE HANDS OF THE COMMITTEE MEMBERS BY DECEMBER 1, 1952.

The offices to be filled and those now holding these offices are as follows:

President-Elect, Mary Nix; Recording Secretary, Sister Charles Miriam (Strassel) (one year term, not eligible for re-election, serving second term); Board of Directors, Mary Frances James and Lucile Harris. Please send your communications at ONCE to: Constance Lee Peterkin, M.T. (ASCP) 928 Manor Rd., Alexandria, Va. (Chairman); Eleanor Fulton, M.T. (ASCP) 537 Millard, Saginaw, Michigan; Lucile Harris, M.T. (ASCP) 1442 N. 3rd St., Abilene, Texas; Marguerite Pitinga, M.T. (ASCP) Memorial Hospital, Colorado Springs, Colorado; Arlene Parks, M.T. (ASCP) 434 Grand Ave., Dayton, Ohio; Doris Boon, M.T. (ASCP) 1588 Ansel Road, Cleveland 6, Ohio.

Constance Lee Peterkin, M.T. (ASCP), Chairman

PROGRAM COMMITTEE

We wish to express our appreciation for the prompt answers which we have already received from some of the State Presidents in response to our letter to them concerning the 1953 Convention Program. May we hope that many members of ASMT will present papers at the 1953 convention in Louisville.

AVAILABLE AWARDS

1. ASCP Registry Award
2. ASMT Convention Awards for scientific papers and scientific exhibits
3. Parasitology Award of $25.00

At this time we do not know if the Hillkowitz Memorial Award will be available this year.

PROGRAM RULES

1. The deadline date for papers to be received by the Program Committee from individuals desiring to present papers in Louisville and likewise to compete for the Convention Awards is March 15, 1953.

2. Only ASMT members are eligible to complete for Convention Awards, except the Registry Award, for which all registered medical technologists are eligible. All competitive papers must be presented in person or by proxy at convention time.

3. All papers read or submitted to the Society become the property of ASMT and may be published in the American Journal of Medical Technology.

4. The time limit for reading the paper on the program is 20 minutes exclusive of showing slides—the remainder of approximately 30 minutes to be given to discussion. Papers may be longer and abstracted for program purposes.

5. All audio visual aids and professional technicians to operate them will be supplied by the Speakers Supplies Committee. Standard lantern slides (3½ by 4) will be furnished unless otherwise requested. Please indicate what aids are needed for the presentation of the paper.

6. FIVE (5) copies of your manuscript must be submitted to the Program Committee Chairman. These must be typewritten, double-spaced, on regular sized typewriter paper.

7. TWO (2) copies of the manuscript must be submitted by all those not competing for ASMT Awards and subject to the above instructions.

8. Prize Papers from state contests to be considered for presentation and further awards must be in the hands of the Program Committee by March 15, 1953.

9. When a paper is submitted for possible awards, place a fly-leaf sheet over the title page containing the following information: Name, title of the paper, award for which the member is competing, whether or not the work represents original research, and whether or not the paper has been published. The AJMT is a copyrighted journal and cannot accept manuscripts which have been published elsewhere. The name of the author should appear **only** on the fly-leaf sheet.

PROGRAM COMMITTEE

Sister Mary Simeonette Savage, 851 South 4th St., Louisville, Ky.
Miss Rachel Lehman, 3939 N. Capital, Indianapolis, Indiana.
Miss Nila Maze, 6106 Carvel, Apt. C 3, Indianapolis, Indiana.
Miss Sarah Hanna, 1439 East Third Street, Salem, Ohio.
Miss Ruth Brumlein, 36 West Crittenden Ave., Ft. Wright, Kentucky.
Mr. Ed Crowe, Health Service, University of Kentucky, Lexington, Ky.

CONCERNING LETTERS TO CONGRESSMEN

By WALTER H. JUDD, Member of Congress from Minnesota

It is commonly said that in a democracy decisions are made by a majority of the people. Of course, that is not true. Decisions are made by a majority of those who make themselves heard.

Good citizenship includes persuading high-grade persons to run for public office, supporting them during campaigns and at the polls and getting others to do likewise. For some it means willingness themselves to become candidates.

But this is not enough. The good citizen must also make himself heard, especially on issues which develop after an election and were not debated in it.

At a time when so many powerful groups are organized to put pressure on Congress to serve their own ends, it is all the more important that your representatives be kept informed on the thinking of those who have no personal axes to grind but desire only what they believe best for the general welfare.

Things Your Congressman Likes

1. He likes to hear opinions from home and wants to keep informed of conditions in the district. Base your letter on your own pertinent experiences and observations.

2. If writing about a specific bill, describe it by number or its popular name. Your Congressman has thousands of bills before him in the course of a year and cannot always take time to figure out to which one you are referring.

3. He likes intelligent, well-thought-out letters which present a definite position, even if he does not agree.

4. Even more important and valuable to him is a concise statement of the reasons for your position, particularly if you are writing about a field in which you have specialized knowledge. He has to vote on many matters with which he has had little or no first-hand experience. Some of the most valuable help he gets in making up his own mind comes from facts presented in letters from persons who really know what they are talking about.

5. Short letters are almost always best. Members of Congress receive many, many letters each day, and a long one may not get as prompt a reading as a brief statement.

6. Letters should be timed to arrive while the issue is alive. If your Congressman is a committee member, he will appreciate having your views while the bill is before him for study and action.

7. A Congressman likes to know when he has done something of which you approve. He is quite as human as you.

THINGS YOUR CONGRESSMAN DOES NOT LIKE

1. He does not like letters that merely demand or insist that he vote for or against a certain bill; or that tell him what you want him to vote for but not why. He has no way of knowing whether your reasons are good or bad, and he is not greatly influenced.

2. He does not like to be threatened with promises of defeat at the next election.

3. He does not like to be told how influential the writer is in his own locality.

4. He does not like to be asked to commit himself on a particular bill as the best means of achieving a desired end, until the committee in charge of the subject has had a chance to hear the evidence and dig out all the pros and cons.

5. He does not like form letters or letters which include excerpts from other letters on the same subject.

6. He does not like to hear from people from other districts, except when the letter deals with a matter which is before a committee of which he is a member. Congressional courtesy makes him refer letters from non-constituents on other subjects to the proper persons.

7. He does not like to be deluged by letters from the same person on the same subject. Quality, not quantity is what counts.

* By permission of Hon. Walter H. Judd.

The above is printed to bring to technologists' attention some of the things necessary to make effective a letter to state or national Congressmen.

—Virginia Burris, M.T., (ASCP) Chairman,
Legislation Committee of A.S.M.T.

AMERICAN ASSOCIATION OF BLOOD BANKS

3500 Gaston Avenue, Dallas, Texas

The Fifth Annual Meeting of the American Association of Blood Banks held in Milwaukee, Wisconsin, October 9, to 11, 1952, attracted well over 500 blood bankers and others interested in the field from all parts of the United States, Italy, India, Puerto Rico, and Germany.

One of the features of the meeting was a Refresher Course for Technicians held the first day. The course, which offered an opportunity for medical technologists and nurses engaged in blood banking to review and compare technical methods, met with enthusiastic interest. It is anticipated that another Refresher Course will be included in the program for next year.

At the business session held on Thursday, October 9th, 1952, the following new officers and board members were elected:

President-Elect

DR. AARON KELLNER, Director of the New York Hospital Blood Bank, New York City and Professor of Clinical Pathology at Cornell University Medical School.

Vice-President

DR. MERLIN L. TRUMBULL, Director of Laboratories, Baptist Memorial Hospital, Memphis, Tennessee.

Treasurer

MRS. BERNICE HEMPHILL, Managing Director, Irwin Memorial Blood Bank, San Francisco, California (re-elected)

Secretary

MISS MARJORIE SAUNDERS, Director of Public Relations, Baylor

Hospital, Dallas, Texas; Secretary, Texas Association of Blood Banks (re-elected)

Director—District 1
DR. JOHN B. HOLYOKE, Director, Community Blood Bank, Mary Hitchcock Memorial Hospital, Hanover, New Hampshire.

Director—District 3
DR. OSCAR B. HUNTER, JR., Director, Oscar B. Hunter Memorial Laboratory, Washington, D. C.

Director—District 5
DR. KARL S. KLICKA, Director, St. Barnabas Hospital, Minneapolis, Minnesota.

Director—District 7
DR. J. RICHARD CZAJKOWSKI, Director, King County Central Blood Bank, Seattle, Washington.

At the conclusion of the annual banquet on October 10th DR. ISRAEL DAVIDSOHN was inducted as President of the Association for the ensuing year. DR. DAVIDSOHN is the immediate past-president of the American Society of Clinical Pathologists. He is presently Director of the Department of Pathology, Mount Sinai Hospital, Chicago, Illinois; Chairman of the department and professor of Pathology, Chicago Medical School; and Director of Research, Mount Sinai Medical Research Foundation, Chicago.

The Sixth Annual Meeting of the Association will be held in Chicago, October 17-20, 1953, at the La Salle Hotel.

IN MEMORIAM
Ralph D. Miller

The immediate Past-President of the Idaho Society of Medical Technologists died suddenly on the morning of August 18, 1952. In his passing the words of his co-workers in that Society pay him the highest tribute. "The Society and its goal for Medical Technology were his prime interests, and there wasn't much he didn't do to help the best way he could. We will all feel his loss more than mere words can describe."

As a delegate to the National Convention in Portland, Oregon, and as an appointee to serve on the ASMT Service Fund and Finance Committee, Mr. Miller was on his way to be a participant in the work of the American Society of Medical Technologists when he died. May we extend to Mrs. Miller our deepest sympathy in her loss.

AMONG THE NEW BOOKS

LABORATORY TECHNIQUE IN BIOLOGY AND MEDICINE, by E. V. Cowdry, Research Professor of Anatomy and Director Wernse Cancer Research Laboratory, Washington University, St. Louis. Third edition, Baltimore, The Williams and Wilkins Co., 1952, 382 pages.

This is a handy reference book for the less routine procedures. Very brief accounts of techniques and a reference source to others are given in alphabetical arrangements. It has the make-up of a dictionary. The key techniques are written by many investigators whose addresses are given so that further information may be obtained directly from the writer. The dates of the last improvements are given, making this an invaluable aid in the last-minute techniques in use.

Emma Cushing, M.T. (ASCP)

A TEXT BOOK OF CLINICAL PATHOLOGY, edited by Seward E. Miller, M. D., Medical Director, United States Public Health Service Chief, Division of Occupational Health, Washington, D. C. (Formerly edited by Roy R. Kracke and Francis P. Parker). Fourth edition, Baltimore, The Williams and Wilkins Company, 1952. 1049 pages.

This book gives the routine procedures and interpretations of laboratory reports as used by the medical student, intern, resident or practicing physician. The more complicated procedures are not given in detail. New chapters in bacterial, viral and rickettsial diseases have been added. The index is somewhat limited and consequently hard to use.

Emma Cushing, M.T. (ASCP)

BIOCHEMISTRY AND HUMAN METABOLISM, by Burnham S. Walker, M. D., Ph. D., Professor of Biochemistry, Boston University School of Medicine; William C. Boyd, Ph. D., Professor of Immunochemistry, Boston University School of Medicine; and Isaac Asimov, Ph. D., Assistant Professor of Biochemistry, Boston University School of Medicine. New, first edition 1952, 812 pages. Baltimore, The Williams & Wilkins Company.

This book is intended primarily for the use of medical students in the study of biochemistry. Much organic chemistry has been avoided in order to leave more space for a discussion in greater detail of the clinical applications of biochemistry. Human biochemistry has been emphasized. The authors have attempted to leave the impression that biochemistry is an expanding science that may and does change rapidly. Although primarily for medical students, this book holds much of interest and scientific information for the medical technologist.

Emma Cushing, M.T. (ASCP)

METABOLIC METHODS. CLINICAL PROCEDURE IN THE STUDY OF METABOLIC FUNCTIONS, by C. Frank Consolazio, Chief of Biochemistry, United States Army Medical Nutrition Laboratory, Chicago, Ill.; Robert E. Johnson, M. D., D. Phil. (Oxford), Professor and Head of the Dept. of Physiology, University of Illinois, Urbana, Ill., and Evelyn Marek, B. A., Biochemist, United States Army Medical Nutrition Laboratory, Chicago, Ill., first edition, 1951, 471 pages, illustrated. St. Louis, The C. V. Mosby Company, 1951. Price, $6.75.

This book is principally one of methods and presents in detail those which have proved useful in the study of man's metabolism in health and disease. The methods most satisfactory for use with a large number of samples to be run routinely have been chosen. The authors make note of the fact that in some instances it was necessary to sacrifice some specificity in favor of simplicity, and sometimes to sacrifice some accuracy for the sake of speed and reproducibility. However, the authors have included only methods which they have used routinely long enough to prove their usefulness. Every method is presented in the same outline form which makes for a very clear description. A list of important precautions are included for each method. There is an excellent section on instrumentation with discussions of the general principles of spectrophotometry, the design of individual machines and calibration work.

Emma Cushing, M.T. (ASCP)

THE STORY OF THE ADAPTATION SYNDROME, Hans Selye, Professor and Director of the Institute de Medecine et de Chirurgie Experimentales, Universite de Montreal, 225 pp., figs. 45, Acta, Inc., Montreal, Canada, 1952, $4.50.

The story is told in the form of informal illustrated lectures. The first lecture is concerned with what made the author think of the adaptation syndrome in the first place and his experience as a very young medical student at the German University of Prague. Patients were presented to them in the earliest stages of various infectious diseases. The patients felt ill and looked ill, and had certain symptoms, but the students were told that these symptoms were of very little significance, and that it was the few "characteristic" signs which would subsequently appear which might help in the diagnosis of specific diseases.

The author, only a novice, was impressed by the fact that only a few signs are actually characteristic of any one disease. Most of them are common to many wholly unrelated maladies—or even to all diseases. He asked himself why it was that widely different pathogens as those of measles, scarlet fever or influenza share with a number of drugs, allergens, etc., the properties of producing "non-specific syndrome," but he learned

that they do and to such an extent that at an early stage a differential diagnosis might even be possible.

However, the author at that time was only an 18-year old medical student, and had neither the training nor facilities for pursuing such thought further until 10 years later in 1935, he was again confronted with this question but under entirely different circumstances.

In this book he tries to reconstruct the story of the adaptation syndrome from the moment its outlines were first faintly suggested by a few incidental observations, and carries us to the present day when it has grown into one of the most complete and most rapidly developing branches of contemporary medicine. He then goes into the first use of the term "stress" and his investigational work after being entrusted with the departmental research program. In his research he has all the usual shattered dreams and disappointments of all research workers. He has some advice for all young people in his audience and for recently graduated physicians who feel they'd like to try their hands at medical research, stating they do not need all the intricate facilities of a modern research institution for their work. One lecture is devoted to the diseases of adaptation, another to the possibility of the same adaptation syndrome producing various diseases. The last chapters are a balance sheet presenting the principal facts, the tangible solid "assets" gained from all the research on stress and the adaptation syndrome. He states that the most important contribution of research on stress is that it has furnished an objective, scientific basis for the development of a new approach to the treatment of disease.

Esther Lemont, M.T. (ASCP)

MICROBIOLOGY, by Florene C. Kelly, M. S., Ph. D., Associate Professor of Bacteriology, the University of Oklahoma School of Medicine, formerly Assistant Professor, Department of Biology, Simmons College, and K. Eileen Hite, Ph. D., M. D., formerly Assistant Professor of Bacteriology, the University of Chicago.

This textbook is a comprehensive study of general and pathogenic microbiology. The first half of the book is devoted to a general study of microorganisms: 1. Introduction to the Microscopic World; 2. Methods for Studying Microorganisms; 3. The Physiology of Bacteria; 4. Microbial Populations; 5. Antimicrobial Methods. The second half of the text, of especial interest to the Medical Technologist, includes: 6. Microorganisms and Disease; 7. Pathogenic Microbiology; 8. Epidemiology and Public Health.

This publication is especially good for its excellent illustrations of the morphology of microorganisms, cultural characteristics and microbiological techniques and equipment. In the appendix there is included a list of common staining methods with formulae for stains and staining procedures.

The appendix also includes a very valuable list of culture media with directions for preparation and complete directions for tests of biochemical activities of microorganisms. The student technologist should find this publication a very good textbook and of equal value as a reference book when doing bacteriology and similar work in the clinical laboratory.

Eileen Darnell, M.T. (ASCP)

ABSTRACTS

SURVEY OF CLOTTING TESTS FOR COAGULATION PROFILE IN NORMAL AND DISEASED PERSONS. Irving A. Friedman, M. D., Steven O. Schwartz, M. D., and Mary Vincenti, Chicago, Ill., Journal American Medical Association, 150 (2): 83-86, Sept., 1952.

Interested in the influence of certain diseases on the clotting mechanism, the authors made a survey of the various clotting tests to obtain the coagulation profiles of normal persons as well as of patients with carcinoma, tuberculosis, leukemia, lymphoma, thrombocytopenia, polycythemia, and aplastic anemia. The Lee-White, heparin retardation, and protamine heparin titration clotting times, the prothrombin values; and red cell, hemoglobin, and platelet determinations of each patient were done for

correlation in this survey. The technics of the procedures employed to draw the profile are given in detail in this paper. Tests were done on normal controls in parallel with the study as often as was thought necessary.

A very large amount of variation in the range of values was found in all groups but especially in the normal persons. The most consistently abnormal results were found in patients with so-called splenogenic thrombocytopenia.

Of the 8 patients with the splenogenic type of thrombocytopenia 3 had very prolonged heparin retardation and protamine titration values which became normal after splenectomy even though 2 patients carried persistently low platelet counts and bled after splenectomy; whereas, another patient displayed prolonged heparin retardation times on 2 occasions after splenectomy without bleeding. These tests showed great variation and no correlation with the platelet counts in the other types of thrombocytopenia.

In the polycythemic group, one patient with a high hemocrit and platelet count showed extreme clotting prolongation on several occasions. The others had normal heparin retardation and variable protamine titrations.

Three of the five patients with aplastic anemia showed prolongation of the heparin retardation time with incoagulability of blood for more than twenty four hours but with no clinical signs of bleeding.

An interesting result was observed in patients with diseases characterized by extensive tissue breakdown as in advanced carcinomia and tuberculosis in that they showed no significant constant change in their coagulation profile.

Because of the wide variation in values obtained in both normal and diseased persons, the authors believe that the tests mentioned, "serve little, if any, useful function in evaluating the clotting mechanism, especially in relation to bleeding and clotting tendencies. The relationship of the platelet count and the bleeding and clotting mechanism awaits further elucidation."

Elsie Berousek, M.T. (ASCP)

A DILUTING FLUID FOR COUNTING ERYTHROCYTES WHICH SIMULTANEOUSLY STAINS THE CELLS AND MAKES THEM LIE FLAT WITHIN A SINGLE FOCAL PLANE. Overdo T. George, Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada. Journal of Laboratory and Clinical Medicine. Vol. 40, No. 3: 479-483, Sept., 1952.

In 1922 Waugh described a technique for using eosin for staining erythrocytes to facilitate their enumeration. However, his method differed in almost all other respects from all those commonly used. The author reports, however, the addition of eosin only, to a conventional diluting fluid.

The composition of the fluid is as follows:

3 per cent aqueous sodium citrate	100.0 ml.
Formalin (commercial, 40 per cent)	1.0 ml.
Water-soluble, bluish eosin	0.6 Gm.

(Eosin, color index 348, obtained from Messrs. G. T. Gurr, London, Eng.)

After it has been filtered, the mixture is ready for use and will keep indefinitely.

A large number of red cell counts were made of the peripheral blood from men and women as well as from various laboratory animals using the eosin-containing diluting fluid. All these counts were made in the usual routine manner. Counts were also made on the same bloods using a conventional diluting fluid.

Actual results of counts made on both normal and pathological bloods showed the range was similar to that of the conventional fluids, however, the actual values are higher ($p < 0.001$) with the eosin-containing fluid. This increase in actual value is supported by observations that have been made with the phase-contrast microscope. There was no evidence of hemolysis and the spherocytes were easily distinguishable in hemolytic anemia blood specimens. For some reason all the cells lay flat and were more uniformly distributed in the counting chamber. Since the cells all lay in one plane

refocusing was not necessary. This increased the speed with which counts could be made and lessened eye-strain and fatigue. The cells stained red and the fluid or background, is pink, thereby making it almost impossible to confuse the cells with debris. With the cells laying flat in one focal plane their relationship to the rulings of the chamber can be more readily determined.

All the advantages of the eosin-containing diluting fluid when used with human blood are present also when it is used for red cell counts on laboratory animals.

Elsie Berousek, M.T. (ASCP)

BIOLOGICALLY FALSE POSITIVE SEROLOGIC TESTS FOR SYPHILIS
(Type, Incidence and Cause)

JOSEPH EARLE MOOREM, M.D., and CHARLES F. MOHR, M.D.
J.A.M.A., Vol. 150, No. 5; 467-473, October 4, 1952

The Wasserman test for the diagnosis of syphilis was introduced into clinical practice in 1907. Since that time this test, which utilizes the phenomenon of complement fixation, has been modified, refined, and simplified through the use of flocculation techniques. Many improvements of the original techniques have been made by various workers giving us new tests bearing the name of its originator—i.e., Kolmer, Kline, Mazzini, Hinton, Eagle, and Kahn and variants of these tests such as the Kahn standard, presumptive and verification tests. While each author-serologist claims his test has some special virtue all of them depend basically on identical physiochemical and immunologic factors, though they differ a little in some minor detail technically. Also, the authors state, that contrary to the general impression all these tests are non-specific.

Non-Specificity of Standard Serological Tests

Antigen is first reviewed and we are reminded that the active antigenic component of beef heart extract (the usual antigen used in standard serodiagnostic tests for syphilis) is a chemically definable substance, cardiolipin. Also, while in infectious diseases other than syphilis, antigens that bring forth antibody response in vivo are directly extracted from the particular etiological virus or organism, but the antigens used in standard serodiagnosis of syphilis are not derived from T. pallidum but from tissue extracts of animals usually not susceptible to infection with T. pallidum. Therefore, considered in the usual immunologic sense, these antigens are non-specific. In review of the antibody, they state that the antibody found in syphilitic serum from these nonspecific antigens is called "reagin," and that little is known of its immunochemical constitution except that its molecular weight is similar to that of other antibodies, and it is associated with the beta and gamma globulin fractions of serum. However, it is

known to be present in the serum of all normal human beings in minute amounts, usually so small that it isn't detected by standard serological tests. "Unfortunately also, reagin (or a reagin like substance) may increase in amount in the serum of non-syphilitic persons in varying incidence ranging from 5 to 100%, during or after a number of infections with no etiological relation to syphilis—enough reagin may be present in serum to produce positive tests for syphilis with standard techniques. The tests in these cases are spoken of as biologically false positive tests."

The mass blood testing program that has been developed in the United States since 1938 has shown that biologically false positives are much more frequent than had been supposed. The results of premarital, prenatal, and pre-employment blood tests; the Armed Forces routine of blood tests on entering and on separation from the Service, plus the routine serological testing of all patients entering most of our best hospitals, and all patients in the practice of many physicians have provided much information on this question. Up to the present no group of positive reactors (except lepers) have been checked with the treponemal immobilization test and so the actual incidence is not yet known. However, of the 75,000 persons (in service) found to be seropositive (with no record of, or clinical evidence of any venereal infection, and no record of any anti-syphilitic treatment), spot surveys of many of this group who were rechecked as seropositive (to exclude technical error) "less than one-half probably had syphilis and more than one-half probably had biologically false positives."

The authors divide the biologically false positive reactions into two types: (1) acute, "characterized by the fact that they occur during or shortly after a wide variety of unrelated non-syphilitic infections or conditions and that they disappear spontaneously within a few days, weeks or months (not more than 6 months) after recovery." These are believed to be caused by bacterial, pneumonia; viral, measles; plasmoidal, malaria; protozoal, trypanosomiasis; spirochetal, relapsing fever; lupus; rheumatoid arthritis; pregnancy and blood loss (multiple donations of blood for transfusions). (2) The chronic type is characterized by the absence of known precipitating factors as found in the acute group and the fact that the reagin persists in the blood over many months or years or even a lifetime. However, leprosy is known to produce a chronic biologically false positive, otherwise its etiology is not clear.

Complicated, time consuming, and expensive procedures are being used, i.e.—examination of spinal fluid (false positive serologic reactions do not occur in spinal fluid) as well as epidemiological investigation of familial and sexual contacts, in trying to reach accurate clinical recognition of biologically false positive reactions.

The results of work started by Turner in 1939 regarding the immunity of syphilis and carried on by Nelson and Mayer proved the existence of a second antibody in syphilitic serum—a "treponemal immobilizing antibody." Further studies originating in Johns Hopkins and confirmed by other workers provided much more information regarding this antibody—it isn't found in the serum of normal people; occurs uniformly in syphilis and related treponemal disease, yaws, pinta, etc.; in untreated cases it parallels the appearance of reagin; in treated cases it disappears from blood as does reagin but more slowly, though not necessarily parallel; in treated late cases it usually doesn't disappear though reagin may do so.

This data, along with others is used as the basis for a tentative conclusion that the treponemal immobilizing tests detect an antibody specific for syphilis and related treponematoses and "it may be expected in a modified form, eventually to replace 'standard' serological tests that depend on lipoid antigens for the detection of non-specific reagin."

The authors use the treponemal immobilization test to differentiate the true syphilitic from the chronic biologically false positive syphilitic where Nelson applied it to the acute type. Results in the latter group were negative in the 12 cases that were used, the false positive was due to 6 cases of primary atypical pneumonia, malaria—1, infectious mononucleosis—1, chicken pox—2 and measles—2. In the former group the test was negative in approximately 84% of the biologically false positives (probable). The authors state that much more work will need to be done before an accurate interpretation of the reason for chronic biologically false positive reactions can be made but that this reaction is usually a manifestation of serious underlying disease. Every effort should be made to differentiate between the biologically false positive reaction and syphilis and if the former is identified the patient should be carefully examined and followed in order to determine the cause.

Elsie Berousek, M.T. (ASCP)

CALENDAR OF MEETINGS AND EVENTS

American Medical Association, New York, N. Y., June 1-5, 1953
International Congress of Microbiology, Rome, Italy, Sept. 6-12, 1953
International Physiological Congress, Montreal, Canada, Aug. 31-Sept. 4, 1953

World Conference on Medical Education, British Medical Association House, London, England, Aug. 24-29, 1953
American Association of Medical Clinics, Denver, Colo., Nov. 30-Dec. 1, 1953

American Society of Tropical Medicine and Hygiene, Galveston, Texas, Nov. 13-15, 1952

Post-Graduate Assembly in Endocrinology and Metabolism, Miami Beach, Florida, Nov. 3-8, 1952
Southern Medical Association, Miami, Florida, Nov. 10-13, 1952

20 cmm. Sahli Pipettes
Tolerance

When calibrating 20 cmm. Sahli Pipettes at the National Bureau of Standards, a tolerance of plus or minus five (5) percent (±5.0%) will be used beginning January 1, 1953. To pipettes found to be within this tolerance range, there will be applied the mark:

NBS
1953

Should a submittor request that a different tolerance range (e.g., smaller) be used in the calibration of a particular lot of pipettes, such request will be complied with.

From correspondence from H. S. Bean, Chief, Capacity, Density and Fluid Meters Section, Mechanics Division, National Bureau of Standards, U. S. Department of Commerce.

ACKNOWLEDGMENT OF GUEST EDITORS

The November-December issue of the AMERICAN JOURNAL of MEDICAL TECHNOLOGY has been edited by the members of the OKLAHOMA SOCIETY of MEDICAL TECHNOLOGISTS. This issue is the INDEX NUMBER—which is not an easy one to prepare.

The Board of Directors of the American Society of Medical Technologists feels that the OKLAHOMA SOCIETY of MEDICAL TECHNOLOGISTS should be complimented on the fine manner in which this State Society has prepared and edited this particular issue.

The Co-ordinator for this issue is Elizabeth Parks, 612 N.E. 12th St., Oklahoma City, Oklahoma.

Sadie Cartwright, M.T. (ASCP)

SCHEDULE OF LABORATORY REFRESHER TRAINING COURSES

January 1 to December 31, 1953

Dates	Course No.	COURSES	Duration	Location*
Jan. 5-9	9.40	Laboratory Diagnostic Methods in Veterinary Mycology	1 Week	1
Jan. 12-23	8.72	Serology of Syphilis**	2 Weeks	3
Jan. 26-Feb. 6	8.40	Laboratory Diagnosis of Bacterial Diseases: Part 1. General Bacteriology	2 Weeks	2
Jan. 26-Feb. 20	8.00	Laboratory Diagnosis of Parasitic Diseases: Part 1. Intestinal Parasites	4 Weeks	1
Feb. 2-13	8.72	Serology of Syphilis**	2 Weeks	3
Feb. 9-20	8.41	Laboratory Diagnosis of Bacterial Diseases: Part 2. General Bacteriology	2 Weeks	2
Feb. 24-Mar. 6	8.50	Laboratory Diagnosis of Bacterial Diseases: Enteric Bacteriology	2 Weeks	2
Feb. 24-Mar. 13	8.01	Laboratory Diagnosis of Parasitic Diseases: Part 2. Blood Parasites	3 Weeks	1
Mar. 9-20	8.72	Serology of Syphilis**	2 Weeks	3
Mar. 16-27	8.20	Laboratory Diagnosis of Virus Diseases***	2 Weeks	5
Mar. 16-27	9.37	Laboratory Diagnosis of Virus Diseases***†	2 Weeks	5
Mar. 16-27	8.15	Laboratory Methods in Medical Mycology: Part 1. Cutaneous Fungi	2 Weeks	1
Mar. 16-27	8.55	Laboratory Diagnosis of Tuberculosis	2 Weeks	2
Mar. 30-April 3	9.36	Laboratory Diagnosis of Tuberculosis†	1 Week	2
Mar. 30-April 10	8.16	Laboratory Methods in Medical Mycology: Part 2. Subcutaneous and Systemic Fungi††	2 Weeks	1
April 6-10	9.34	Laboratory Diagnosis of Bacterial Diseases†	1 Week	2
April 6-17	8.72	Serology of Syphilis**	2 Weeks	3
April 13-17	9.33	Laboratory Diagnosis of Parasitic Diseases†	1 Week	1
April 13-17	8.26	Laboratory Diagnosis of Rabies	1 Week	2
April 27-May 1	9.38	Laboratory Diagnosis of Venereal Disease**†	1 Week	4
May 4-8	9.60	Microbiology for Public Health Nurses	1 Week	1
May 4-15	8.72	Serology of Syphilis**	2 Weeks	3
May 18-29	8.74	Preparation and Standardization of Cardiolipin Antigens Used in Serologic Tests for Syphilis**	2 Weeks	3
Aug. 17-28	8.72	Serology of Syphilis**	2 Weeks	3
Sept. 14-25	8.72	Serology of Syphilis**	2 Weeks	3
Sept. 21-Oct. 2	8.40	Laboratory Diagnosis of Bacterial Diseases: Part 1. General Bacteriology	2 Weeks	2
Sept. 21-Oct. 16	8.00	Laboratory Diagnosis of Parasitic Diseases: Part 1. Intestinal Parasites	4 Weeks	1
Oct. 5-16	8.41	Laboratory Diagnosis of Bacterial Diseases: Part 2. General Bacteriology	2 Weeks	2
Oct. 5-16	8.73	Preparation and Use of Controls in Serologic Tests for Syphilis**	2 Weeks	3
Oct. 19-30	8.50	Laboratory Diagnosis of Bacterial Diseases Enteric Bacteriology	2 Weeks	2
Oct. 19-30	8.20	Laboratory Diagnosis of Virus Diseases***	2 Weeks	5
Oct. 19-30	9.37	Laboratory Diagnosis of Virus Diseases***†	2 Weeks	5
Oct. 19-Nov. 6	8.01	Laboratory Diagnosis of Parasitic Diseases: Part 2. Blood Parasites	3 Weeks	1
Oct. 26-Nov. 6	8.72	Serology of Syphilis**	2 Weeks	3
Nov. 2-6	9.34	Laboratory Diagnosis of Bacterial Diseases†	1 Week	2
Nov. 9-13	9.36	Laboratory Diagnosis of Tuberculosis†	1 Week	2
Nov. 9-13	8.26	Laboratory Diagnosis of Rabies†	1 Week	1
Nov. 9-20	8.74	Preparation and Standardization of Cardiolipin Antigens Used in Serologic Tests for Syphilis**	2 Weeks	3
Nov. 16-20	9.33	Laboratory Diagnosis of Parasitic Diseases†	1 Week	1
Nov. 16-27	8.55	Laboratory Diagnosis of Tuberculosis	2 Weeks	2
Nov. 23-27	9.35	Laboratory Methods in Medical Mycology†	1 Week	1
Nov. 30-Dec. 11	8.17	Laboratory Methods in the Study of Pulmonary Mycoses	2 Weeks	1
Dec. 7-18	8.72	Serology of Syphilis**	2 Weeks	3
†††	8.05	Laboratory Diagnosis of Malaria	2 Weeks	1
†††	8.10	Identification of Medically Important Arthopods	2 Weeks	1
†††	8.21	Virus Isolation and Identification Techniques	2-4 Weeks	5
†††	8.25	Laboratory Diagnosis of Influenza	1 Week	5
†††	8.42	Typing of **Corynebacterium Diphtheriae**	1 Week	2
†††	8.51	Special Problems in Enteric Bacteriology	2 Weeks	2
†††	8.52	Phage Typing of **Salmonella Typhosa**	1 Week	2
†††	8.75	Serologic Diagnosis of Rickettsial Diseases	1 Week	2

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